Support for the amendments to claims 1 and 6 may be found *inter alia* in the specification specifically on page 13, lines 16 through page 14, line 11. Moreover, support for "with attenuated DNA binding activity" may be found in Brief Description of Figures for Figures 4 and 5. Applicant maintains that these amendments raise no issue of new matter. Thus, claims 1,4,6-12,15,16,24-28,30-33, and 42-46 will be pending upon entry of this amendment.

INFORMATION DISCLOSURE STATEMENT

In accordance with his duty under 37 C.F.R. §1.56, applicant would like to direct the Examiner's attention to the following documents which are listed below and on the accompanying PTO Form 1449 attached hereto as **Exhibit A** and the International Search Report attached hereto as **Exhibit B**. Copies of these Documents are attached hereto as **Exhibit C-N**.

1. Bednarik, D., J. Cook and P. M. Pitha, *Inactivation Of The HIV LTR By DNA CpG Methylation: Evidence For A Role In Latency, EMBO J.* (1990) **9**(4):1157-64 (**Exhibit C**);

2. Bestor, T. H., *Activation Of Mammalian DNA Methyltransferase By Cleavage Of A Zn-binding Regulatory Domain, EMBO J.* (1992) **11**(7):2611-17 (**Exhibit D**);

3. Brent, R., and Ptashne, M., *A Eukaryotic transcriptional Activator Bearing The DNA Specificity Of A Prokaryotic Repressor, Cell* (1985) **43**(3):729-36 (**Exhibit E**);

4. Busslinger, M., J. Hurst, R. A., Flavell, *DNA Methylation And*

The Regulation Of Globin Gene Expression, Cell (1983) **34**(1):197-206 (**Exhibit F**);

5. Challita, P.-A., and D. B. Kohn , *Lack Of Expression From A Retroviral Vector After Transduction Of Murine Hematopoietic Stem Cells Is Associated With Methylation in vivo, Proc. Nat. Acad. Sci. USA* (1994) **91**(7):2567-71 (**Exhibit G**);

6. Desjarlais, J. R., and J. M. Berg, *Redesigning The DNA-Binding Specificity Of A Zinc Finger Protein: A Database-Guided Approach, Proteins* (1992) **12**(2):101-104 (**Exhibit H**);

7. Joel, P., W. Shao, and K. Pratt, *A Nuclear Protein With Enhanced Binding To Methylated Sp1 Sites In The AIDS Virus Promoter, Nucl. Acids Res.* (1993) **21**(24):5786-93 (**Exhibit I**);

8. Palmiter et al., *Germ-Line Transformation Of Mice, Ann. Rev. Genet.* (1986) **20**:465-99 (**Exhibit J**);

9. Prasad et al., *Domains With transcriptional Regulatory Activity Within The ALL1 And AF4 Proteins Involved In Acute Leukemia, Proc. Natl. Acad. Sci. USA* (1995) **92**(26):12160-64 (**Exhibit K**);

10. Rebar, E. J., and C. O. Pabo, *Zinc Finger Phage: Affinity Selection Of Fingers With New DNA-Binding Specificities, Science* (1994) **263**:671-73 (**Exhibit L**);

11. Wu, H., W.-P Yang, and C. F. Barbas, *Building Zinc Fingers By Selection: Toward A Therapeutic Application, Proc. Nat. Acad.*

Sci. USA (1995) **92**(2):344-48 (**Exhibit M**); and

12. Zhukovskaya et al., *Inactive O^6-methylguanine-DNA Methyltransferase In Human Cells, Nucl. Acid. Res.* (1992) **20**(22):6081-90 (**Exhibit N**).

Applicant requests that the Examiner make these documents of record in the subject application.

Applicant maintains that none of the above listed documents which are citable as a reference against the subject application disclose or suggest the invention now being claimed. Applicant attaches hereto as **Exhibits C-N** copies of the above-listed references. Applicant requests that the Examiner make these documents of record.

No fee, other than the TWO HUNDRED AND FORTY DOLLAR ($240.00) fee under 37 C.F.R. §1.97 is deemed necessary in connection with the filing of this Information Disclosure Statement. However, if any fee is required, authorization is hereby given to charge the amount of any such fee to Deposit Account No. 03-3125.

Restriction Requirement

The Examiner stated that applicant's response to the restriction requirement, filed on 4/10/00, has been acknowledged and that applicant's election of Group I, claims 1-46, and the election of the following species a) chimeric proteins comprising a zinc three-finger DNA binding polypeptide linked to a CpG-specific DNA methyltransferase polypeptide, b) target genes associated with cancer, c) target genes wherein the target gene is endogenous, and d) animals as the multicellular organism, in Paper No. 6 is acknowledged. Additionally, the Examiner stated that because

applicant did not distinctly and specifically point out the supposed errors in the restriction requirement, the election has been treated as an election without traverse(MPEP §818.03(a)).

The Examiner stated that claims 1-47 are pending.

The Examiner stated that claims 2,3,5,13,14,17-23,29,34-41, and 47 are withdrawn from consideration as being drawn to a non-elected invention (claim 47) or species (claims 2,3,5,13,14,17-23,29,34-41) and that claims 1,4,6-12,15,16,24-28,30-33, and 42-46 are being examined on the merits to the extent that the claims read on the elected species.

Applicant notes that a non-elected species may be reintroduced if a generic claim is found allowable (M.P.E.P. §809).

Rejection Under 35 U.S.C. §112, First Paragraph - Written Description

On page 2-3 of the July 6, 2000 Office Action, the Examiner rejected claims 1-10, and 42-46 under 35 U.S.C. §112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

The Examiner stated that these claims are directed to a chimeric protein which comprises a zinc three-finger DNA binding polypeptide linked to a CpG-specific DNA methyltransferase polypeptide, wherein the DNA binding polypeptide binds sufficiently close to a promoter sequence of a target gene, wherein the target gene is an endogenous

gene associated with cancer, wherein the promoter sequence contains a methylation site, and wherein the site is specifically methylated such that activity of the promoter, and thus expression of the target gene is inhibited; vectors comprising a polynucleotide encoding the chimeric protein, cells containing the vector, and pharmaceutical compositions containing vectors.

In analyzing whether the written description requirement is met for genus claims, the Examiner stated, it is first determined whether a representative number of species have been described by their complete structure. In this case, the Examiner alleged that the specification does not disclose a chimeric peptide which specifically targets a promoter of an endogenous gene associated with cancer and with regard to genes associated with cancer, the specification discloses that genes that are overexpressed in cancer cells are target genes of the subject invention and include the cancer related genes collagenase 92 kD Type 4, collagenase 72 KD Type 4, osteopontin, calcyclin, fibroblast growth factor, epidermal growth factor, matrilysin and stromolysin. However, the Examiner alleged, the specification does not disclose the sequences of these promoters to which the chimeric protein should bind, or whether the promoter sequences contain a methylation site which is specifically methylated and which results in inhibition of expression of the genes. Similarly, the Examiner alleged, the specification does not disclose the sequence (amino acid or polynucleotide) required in the zinc three finger binding polypeptide which will allow such targeting specificity.

The Examiner stated that the limited information provided in the specification is not deemed sufficient to reasonably convey to one skilled in the art that applicants were in possession of the chimeric proteins or polynucleotides encoding the chimeric proteins

which have the claimed targeting specificity at the time the application was filed and thus it is concluded that the written description requirement is not satisfied for the claimed genera.

In response, applicant traverses the Examiner's rejection of claims 1-10, and 42-46 under 35 U.S.C. §112, first paragraph and maintains that the claimed invention is fully and sufficiently described in the specification and that one of skill in the relevant art would have understood applicant to have possession of the claimed invention.

With regard to the Examiner's rejection that the specification fails to disclose a chimeric peptide which specifically targets a promoter of an endogenous gene associated with cancer, applicant first directs the Examiner's attention to the applicable standard.

The Written Description standard (see MPEP §2163.02) is an **objective** test for sufficiency of support in a parent application and whether the disclosure of the application relied upon "**reasonably conveys** to the artisan that the inventor had possession at that time of the later claimed subject matter." *In Re Kaslow*, 707 F.2d 1366, 1375 (Fed. Cir. 1983)

Applicant notes that one of skill in the art, provided with guidance from the specification, would be able to practice the claimed invention when combined with what was disclosed in the prior art. At the time of applicant's effective filing date, September 25, 1995, one of skill in the art would have known of many promoter sequences of genes known to be involved in cancer. Specifically, applicant includes Genbank sequences referenced for human fetal liver spleen (Genbank No. 734345); human infant brain (Genbank No. 673889); Chicken Osteopontin mRNA (Genbank No.

211276); Human placenta(Genbank No. 654575); human osteopontin mRNA, complete cds (Genbank No. 189404); Mus musculus osteopontin mRNA (Genbank No. 200157); Rattus norvegicus osteopontin mRNA, complete cds (Genbank No. 205867); Rat Osteopontin mRNA, complete cds (Genbank No. 205859); Mouse osteopontin (Genbank No. 200159); Sus scrofa osteopontin gene (Genbank No. 164599); Bovine Osteopontin (Genbank No. 162890); and Gallus domesticus osteopontin (Genbank No. 404635) attached herewith as **Exhibit O**.

Applicant reiterates that it is not necessary to include in a patent, information which would be know to one of skill in the art and that is not essential information (M.P.E.P. §2141).

One of skill in the art at the time of applicant's effective filing date, would have known various DNA binding proteins which were known to specifically bind to specific DNA sequences involved in cancer. DNA binding proteins which bind to promoter sequences found in the promoters of cancer-related genes were also known. For example, the DNA-binding protein, MDBP binds specifically to a site in the beginning of the first intron of the human c-myc gene (Zhang Y., et al. Cancer Research (1990) 50(21):6865-9); The DNA-binding protein MIBP1 showed sequence-specific binding to the target sequence rat c-myc intron 1 (Makino r., et al. Nucleic Acids Research (1994) 22(25):5679-85); The DNA-binding protein MSSP-2 has been cloned and its binding specificity characterized (Takai, T., et al. Nucleic Acids 22(25):5576-81); and the characterization and binding activity of the full-length GST-A-myb fusion protein interacts with specific binding sites on the c-myb responsive promoters, MIM-1 and CD34 (Ma X. P., et al, (1994) Cancer es. 54(24):6512-6).

As of the effective filing date, one of skill in the art would have

known how to recognize a methylation site in a promoter sequence. Indeed, applicant's specification teaches that the CpG sequence in promoters is such a putative methylation site (specification at page 8, legend of Figure 12). It would have been routine for one of skill in the art to test whether a target promoter sequence contains a methylation site, and if it does, provided with guidance from applicant's invention, inhibit the expression of a gene of interest because one of skill in the art would have known that **all** mammalian promoters tested to date have been found to be silenced when they contain 5-methylcytosine (m^5c) at CpG sites (reviewed by Bestor, 1990; Meehan et al., 1993).

Additionally, applicant provides evidence for distribution of CpG sites for targeted methylation and gene inactivation (see specification at page 5, legend of Fig. 1, 2 & 3; spec. at page 8. legend of Fig. 11A - 11B). Moreover, applicant providence support for selection for DNA binding protein/DNA methyltransferase chimeras in which methylation is dependant on the DNA binding moiety (see spec. at page 7, legend of Fig. 8)

Applicant emphasizes that they are claiming **a chimeric protein** and provide a disclosure which enables the skilled artisan to make and use the chimeric protein. Applicant maintains that whether a chimeric protein is chosen that target genes associated with cancer, AIDS, a central nervous system disorder, a blood disorder, a metabolic disorder, a cardiovascular disorder, an autoimmune disorder or an inflammatory disorder is irrelevant to whether applicant's **invention** is described. Applicant provides detailed teachings to make and use the claimed chimeric protein, and one of skill in the art would have known of many promoter sequences of genes known to be involved in cancer, one of skill in the art would have known various DNA binding proteins which were known to

specifically bind to specific DNA sequences of differing cancer types and because one of skill in the art would have known how to recognize a methylation site in a promoter sequence associated with cancer.

In light of the above, applicant requests that the Examiner reconsider and withdraw the above ground of rejection.

Rejection Under 35 U.S.C. §112, First Paragraph - Enablement

On pages 4-8, the Examiner rejected claims 1,4,6-12,15,16,24-28,30-33, and 42-46 under 35 U.S.C. §112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

The Examiner stated that claims 1-10, and 42-46 are directed to a chimeric protein which comprises a zinc three-finger DNA binding polypeptide linked to a CpG-specific DNA methyltransferase polypeptide, wherein the DNA binding polypeptide binds sufficiently close to a promoter sequence of a target gene, wherein the site is specifically methylated such the activity of the promoter, and thus, expression of the target gene is inhibited; vectors comprising a polynucleotide encoding the chimeric protein, cells containing the vector, and pharmaceutical compositions containing the vector.

The Examiner stated that claims 11,12,15,16, and 24-26 are directed to a method for inhibiting expression of an endogenous target gene associated with cancer in an animal comprising contacting a promoter sequence of the target gene with the chimeric protein to

specifically methylate the promoter sequence resulting in inhibition of expression of the target gene.

The Examiner stated that claims 17, 28, and 30-33 are directed to a method for inhibiting expression of an endogenous target gene associated with cancer in an animal comprising contacting a promoter sequence of the target gene with the chimeric protein to specifically methylate the promoter sequence resulting in inhibition of expression of the target gene in the animal.

The Examiner stated that the specification discloses specific methods for selecting zinc finger proteins that bind to predetermined sequences in the HIV-1 5' LTR, and methods of inactivating HIV in cells *in vitro*. However, the Examiner alleged that the specification does not disclose any predetermined sequences in target endogenous genes associated with cancer, or any specific zinc finger DNA binding polypeptide which would selectively bind to the targeted genes such that specific sites on the promoters of the targeted genes are methylated, resulting in inactivation of the promoters and inhibition of gene expression.

The Examiner acknowledged that the specification indicates that cancer related genes of interest include collagenase 92 KD Type 4, osteopontin, calcyclin, fibroblast growth factor, epidermal growth factor, matrilysin, and stromolysin. However, the Examiner alleged the specification does not provide any "predetermined sequences" associated with the regions of these promoters such that selection of appropriate zinc finger proteins could be achieved, or whether these particular promoters have specific sites which can be methylated and thereby inactivated such that expression of the gene is inhibited. Moreover, the Examiner alleged, the specification does not provide any amino acid sequence of a chimeric protein or

polynucleotide encoding the chimeric protein which has the claim-designated properties. The Examiner concluded that given the alleged lack of guidance in the specification as to a particular chimeric protein having the claim-designated properties, or methods for specifically making the chimeric protein having the claim-designated properties, one of skill in the art would be able to make the chimeric protein without undue experimentation.

The Examiner also alleged that with regard to using the chimeric protein to methylate specific sites on a promoter, thereby inactivating the promoter and inhibiting expression of the endogenous gene associated with cancer, the specification does not disclose any specific chimeric protein to be used in the method, nor does the specification disclose how to administer the protein, *per se*. For example, the Examiner stated that there is no teaching of the amount of chimeric protein to administer, or the site of administration. In addition, the Examiner stated, there is no teaching in the specification of endocytosis of the protein such that a sufficient amount of protein is incorporated into the cell, transported to the nucleus, and binds to the appropriate target gene. The Examiner stated that because the specification does not disclose a particular chimeric protein with the claim-designated properties or a method of administrating the protein, per se., it would require undue experimentation for one of skill in the art to use the chimeric protein as claimed.

The Examiner stated that with regard to methods of contacting a promoter with the chimeric protein via a vector comprising a polynucleotide encoding the chimeric protein, the methods encompass gene therapy. However, the Examiner stated, the specification does provide sufficient guidance in the specification with respect to the amount of vector to administer, the route of administration,

the required expression level of the vector, the tissue to be targeted such that the chimeric protein is synthesized in the appropriate cell at a sufficient level for binding to the targeted gene, methylating a specific site on the promoter of the gene and inhibiting expression of the gene. Moreover, the Examiner stated, at the time of filing, the art of gene therapy was known to unpredictable and non-routine and that in the "Report and recommendations of the Panel to Assess the NIH Investment in Research on Gene Therapy" (published December 7, 1995), Orkin and Motulsky indicate that clinical efficacy has not been definitively demonstrated at this time in any gene therapy protocol; that major difficulties of gene therapy include shortcoming in all current gene therapy vectors and an inadequate understanding of the biological interaction of these vectors with the host; that it is not always possible to extrapolate directly from animal experiments to human studies; and that while the most straight-forward application of gene therapy may be in the treatment of single-gene inherited disorders, practical difficulties need to be addressed, i.e. delivery of the appropriate gene to a specific cell type or tissue, gaining access to the relevant cell type for correction of the defect, assessing the total fraction of cells in a tissue that need to be corrected, achieving the level of expression required for correction, and regulating expression of the added gene once it is transferred into appropriate target cells (see, e.g., pages 1 and 2, points 2,3 and 5, for example, page 5, under "Single-gene inherited disorders', and page 14, bullet paragraphs 3-6). Similarly, the Examiner Stated, Verma et al. (Nature, 387:239-242, 1997) indicate that "In principle, gene therapy is simple: putting corrective genetic material into cells alleviates the symptoms of disease. In practice, the Examiner stated, considerable obstacles have emerged; problems such as lack of efficient delivery systems, lack of sustained expression, and host immune response reactions

remain formidable challenges; although more than 200 clinical trials are currently underway worldwide, with hundreds of patients enrolled, there is no single outcome that we can point to as a success story" (see page 239, under Abstract, and left column, paragraphs 1-2). The Examiner stated that the obstacles apply to the claim-designated viral vectors, i.e., retroviral, adenoviral, and adeno-associated viral vector delivery systems (see, e.g., pages 240 and 241, under the sections entitled " Retroviral vectors", "Adenoviral vectors", and "Adeno-associated viral vectors"). Furthermore, the Examiner stated, Ledley (Pharmaceutical Research, 13:1595-1614, 1996) discloses that while there is growing confidence that genes therapy will provide important pharmaceutical products, and that clinical trials have demonstrated that genes can be introduced into patients by several different methods and will express potentially therapeutic gene products, significant hurdles remain. The Examiner stated that several recent clinical studies failed to demonstrate the expected pharmacological effects. Moreover, the Examiner stated, some of the methods that have been proposed for gene therapy have limiting toxicities, are difficult to manufacture and quality control, or are more costly than current therapies (see page 1595, right column, second paragraph). The Examiner stated that retroviral vectors can be directly administered to patients, though the applicability of this approach is limited by the rapid inactivation or retroviruses by human complement (see page 1596, right column, last paragraph bridging page 1597). The Examiner stated that the major limitation of adeno-associated viruses has been difficulty in developing packaging cell lines that will produce sufficient titers of the virus for clinical use without the presence of helper virus (see, page 1597, right column, line 1-4). The Examiner stated that adenoviral vectors have been demonstrated to be toxic, inducing cytopathic and immunogenic responses *in vivo* and that

preclinical and clinical studies have been demonstrated that the level and persistence of gene expression using adenoviral vectors may inhibited by the immunological responses against the adenoviral particle, and inflammation in tissues targeted by the vector (see page 1597, right column, last paragraph). Moreover, the Examiner stated that Ledley states that the effectiveness of gene delivery *in vivo* is poorly predicated by *in vitro* results. The Examiner stated that the reasons why *in vitro* results would not be recapitulated *in vivo* include various biological barriers that are not reflected in *in vitro* models, recapitulated in vivo include various biological barriers that are not reflected in *in vitro* models, and interactions between DNA or formulated DNA complexes with serum and blood elements (see page 1603, right column).

The Examiner stated that in view of the lack of guidance in the specification of a particular chimeric protein having the claim-designated properties, the lack of teaching in the specification of how to use the chimeric protein such that the expression of an andogenous target gene associated with cancer can be inhibited via methylation of specific sites on the promoter of the target gene, and the unpredictability in the art at the time of filing of gene therapy, one of skill in the art would not be able to make the chimeric protein, the vector comprising a polynucleotide encoding the chimeric protein, cells comprising the vector, or pharmaceutical compositions comprising the vector or protein, nor use the chimeric proteins or vectors as claimed.

In response, applicant traverses the rejection of claims 1, 4, 6-12, 15, 16, 24-28 30-33 and 42-46 under 35 U.S.C. §112, first paragraph, and maintains that one of skill in the art is fully enabled to make and/or use the claimed invention as claimed.

The standard for enablement is "whether one **reasonably skilled** in the art could make or use the invention from the disclosures in the patent **coupled with information known in the art** without undue experimentation." (see M.P.E.P. §2164.01).

Applicant claims "A chimeric protein which comprises (1) a [mutated] DNA methyltransferase with attenuated DNA binding activity linked to (2) a DNA binding protein that binds sufficiently close to a promoter sequence of a target gene, which promoter sequence contains a methylation site, to specifically methylate the site and inhibit activity of the promoter and thus inhibit expression of the target gene." Applicants emphasize that they are claiming **a chimeric protein** and provide a disclosure which enables the skilled artisan to make and use the chimeric protein. Applicant maintains that whether he chooses chimeric proteins that target genes associated with cancer, AIDS, a central nervous system disorder, a blood disorder, a metabolic disorder, a cardiovascular disorder, an autoimmune disorder or an inflammatory disorder is irrelevant to this rejection. Applicants provide detailed teachings to make and use the claimed chimeric protein.

With regard to the Examiner's assertion that applicant fails to provide predetermined sequences in target genes associated with cancer or any specific zinc finger DNA binding peptide which would selectively bind to the targeted gene resulting in inactivation of the promoters and inhibition of gene expression, applicant maintains that one of skill in the art, provided with guidance from the specification on page 29, line 33 through page 30, line 11 and Example 3 on page 44, could readily make chimeric proteins within the scope of applicant's claims and test which would work and which

would not without undue experimentation. Applicant teaches the construction of a chimeric protein which contains a zinc three-finger DNA binding component and a DNA methyltransferase protein with attenuated DNA binding activity which methylates a specific target promoter sequence. Applicant teaches that methylation of a promoter sequence halts transcription and that **all** mammalian promoters tested to date have been found to be silenced when they contain 5-methylcytosine (m^5c) at CpG sites (reviewed by Bestor, 1990; Meehan et al., 1993).

Applicant directs the Examiner's attention to page 7 of the specification. Therein, applicant provides specific and detailed methods for selection of zinc finger proteins that bind to predetermined sequences in the HIV-1 5' protein (specification at page 7, legend of Fig. 9; specification at page 9, legend of Fig. 15; specification at page 44, Example 3 through page 47, line 12). Applicant maintains that no undue experimentation is required to practice the claimed invention because applicant teaches a protocol for the design, selection and affinity maturation of zinc finger-DNA chimeras that methylate critical CpG sites in the HIV 5' LTR, which can readily be applied to other promoters. Specifically, applicant provides three important factors to be considered in the design and selection of zinc finger-DNA methyltransferase chimeras (specification at page 44, lines 28 through page 45, line 9). Once Zinc finger proteins meet the three factor test, they can be selected from combinatorial expression libraries by phage display and fused to the CpG-specific DNA methyltransferase M.SssI as described in Example 1, and depicted in Figure 9. Applicant provides for the selection of tridactyl zinc finger proteins that bind to predetermined sequences (Figure 9) Applicant provides a detailed map for Zinc Finger selection, construction of a phage library and screening for clones is well known to those of skill in

the art. One of skill in the art, provided with guidance from the specification, need only follow the protocol disclosed to design and create zinc three finger chimeras that methylate specific target sequences.

Moreover, as mentioned above, applicant provides examples of known DNA sequences for which DNA-binding proteins are known to bind. Applicant provides citations of publications which disclose such information, attached herewith as **Exhibit P**. For example, the DNA-binding protein MDBP binds specifically to a site in the beginning of the first intron of the human c-myc gene (Zhang Y., et al. *Cancer Research* (1990) 50(21):6865-9); The DNA-binding protein MIBP1 showed sequence-specific binding to the target sequence rat c-myc intron 1 (Makino r., et al. *Nucleic Acids Research* (1994) 22(25):5679-85); The DNA-binding protein MSSP-2 has been cloned and its binding specificity characterized (Takai, T., et al. *Nucleic Acids* 22(25):5576-81); and the characterization and binding activity of the full-length GST-A-myb fusion protein interacts with specific binding sites on the c-myb responsive promoters, MIM-1 and CD34 (Ma X. P., et al, (1994) *Cancer Res.* 54(24):6512-6).

With regard to the Examiner's assertion that no amino acid sequence of a chimeric protein which has claim-designated properties is disclosed or could be achieved without undue experimentation, applicant points out that one of skill in the art knew on the filing date what a methylation site was and how to determine its presence in a promoter sequence. More importantly, one of skill in the art knew that **all** mammalian promoters tested to date have been found to be silenced when they contain 5-methylcytosine (m^5c) at CpG sites (reviewed by Bestor, 1990; Meehan et al., 1993). Moreover, applicant has cited publications, attached herewith as

Exhibit O, published before applicant's effective filing date, i.e. September 25, 1995, which disclose sequences of target genes to which specific DNA-binding proteins bind. Lastly, applicant teaches the construction of a plasmid that performs targeted methylation (see spec. at page 8, legend of Fig. 12) and includes guidance for the targeted *de novo* methylation of a specific sequence (Example 1, page 39); HIV-1 LTR constructs used to analyze targeted methylation (see spec. at page 10, legend of Fig. 16); the identification of CpG sites in the HIV-1 5' LTR whose methylation produces maximal repression of transcription (Example 2, page 42); and the construction and selection of zinc-finger (Example 3, page 44).

With respect to the Examiner's assertion that the specification does not teach the endocytosis of the protein, applicant maintains that one of skill in the art, given the disclosure in the specification that DNA methylation does suppress transcription (see specification page 4) would be able to construct a chimeric protein to inhibit gene expression. One of skill in the art would only need to create a chimeric protein with a DNA binding protein portion linked to a mutated DNA methyltransferase portion which is capable of binding to a predetermined target promoter sequence with a methylation site to inhibit gene expression. Applicant directs the Examiner's attention to Figure 1 depicting the inactivation of 5' LTR of HIV-1 by targeted cytosine methylation.

With respect to the Examiner's concerns regarding gene therapy problems, applicant maintains that all that is needed to practice is disclosed, the rest is known to those of skill in the art. Applicant provides support in the specification at page 22, lines 6 - page 23, line 4; and page 22, lines 13-21.

In light of the above, applicant requests that the Examiner reconsider and withdraw the above ground of rejection.

Rejection Under 35 U.S.C. §112, Second Paragraph

The Examiner rejected claims 1,4,6-12,15,16,24-28,30-33 and 42-46 under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

The Examiner stated that claim 1 is rendered vague and indefinite by the phrases "mutated DNA methyltransferase portion" and "DNA binding protein portion" as it is unclear what type of mutation is intended, and what a "portion" of a protein represents and that the metes and bounds of the phrases are unclear.

The Examiner stated that claim 6 is confusing because the claim recites " The method of claim 1", however, claim 1 is directed to a chimeric protein, not a method. Clarification is requested. Claim 6 is also rendered vague and indefinite by the phrases "at least a portion of a mutated M.*SSSI* DNA methyltransferase protein" and "at least a portion of a mutated mammalian DNA methyltransferase protein" as it is unclear what mutation is required and which portion of the protein is intended such that the protein has the claim-designated activities. The Examiner stated that the metes and bounds of the phrases are unclear.

In response, but without conceding the correctness of the Examiner's position, applicant has amended claim 1 by deleting the words "mutated" and "portion" and inserting the phrase "with attenuated DNA binding activity linked to (2)". Applicant has amended claim 6 by deleting the words "mutated," "portion" and "at

least a portion of" and inserting the phrases "chimeric protein" and "with attenuated DNA binding activity".

Applicant maintains that the specification discloses the regulatory and catalytic domains of DNA methyltransferases and that the invention would use the catalytic domain of the methyltransferase and not the regulatory domain. Applicant notes that the DNA methyltransferase of Part (1) of claim 1 includes the catalytic domain and not the regulatory domain. Specifically,

> [t]he agents which are embodiments of this invention differ from mammalian DNA methyltransferase in that the regulatory domain targets the catalytic domain to predetermined sequences. The regulatory region of mammalian DNA methyltransferase suppresses the methylation of previously unmethylated sites (Bestor, 1992), and directs the protein to sites of new DNA synthesis in S (synthesis) phase nuclei (Leonhardt et al., 1992). The Targeted methylation which is an embodiment of this invention is grounded in the knowledge of the basic biology of the system that establishes and maintains methylation patterns in the mammalian genome. (specification page 32, line 25 through page 33, line 3).

In addition to Example 1, the subject specification recites that "it is therefore necessary to make the DNA methyltransferase moiety dependent on LexA-mediated DNA binding; this may be accomplished by selection of mutant versions of M.*Sss*I that have reduced intrinsic DNA binding activity. A novel cyclic in vivo/in vitro selection protocol is used to select mutant proteins of the desired character." (specification at page 39, line 20-26). Thus, applicant maintains that the DNA methyltransferase has "reduced intrinsic DNA binding activity", supporting the aforementioned amendments.

Applicant has additionally amended claim 6 to correct an obvious

typographical error. Applicant has canceled the word "method" from claim 6 and replaced it with the word "protein". Applicant maintains that the amendment to claim 6 obviated the Examiner's rejection of said claim under 35 U.S.C. §112, second paragraph.

In light of the above, applicant requests that the Examiner reconsider and withdraw the above grounds of rejection.

If a telephone interview would be of assistance in advancing prosecution of the subject application, applicant's undersigned attorney invites the Examiner to telephone at the number provided below.

No fee, other than the $685.00 fee for the three-month extension ($445.00) and the Information Disclosure Statement ($240.00) is deemed necessary in connection with the filing of this Amendment. However, if any fee is required, authorization is hereby given to charge the amount of any such fee to Deposit Account No. 03-3125.

Respectfully submitted,

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I hereby certify that this correspondence is being deposited this date with the U.S. Postal Service with sufficient postage as first class mail in an envelope addressed to:

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